IDE▲L®

Anisotropy in Tendon Investigated *in Vivo* by a Portable NMR Scanner, the NMR-MOUSE

R. Haken and B. Blümich

Institute for Technical Chemistry and Macromolecular Chemistry, RWTH Aachen, Germany

Ordered tissue like tendon is known to exhibit the magic-angle phenomenon in magnetic resonance investigations. Due to the anisotropic structure the transverse relaxation time T_2 depends on the orientation of the tendon in the magnetic field. In medical imaging, relaxation measurements of tendon orientation are restricted by the size of the object and the space available in the magnet. For humans, tendon orientation can only be varied within small limits. As a consequence, the magic-angle phenomenon may lead to a misjudgement of tendon condition. It is demonstrated that the NMR-MOUSE (mobile universal surface explorer), a hand-held NMR sensor, can be employed to investigate the anisotropy of T_2 in Achilles tendon *in vivo*. The NMR-MOUSE provides a convenient tool for analyzing the correlation of T_2 and the physical condition of tendon. © 2000 Academic Press

Key Words: tendon; relaxation; NMR-MOUSE.

INTRODUCTION

From different investigations it is known that anisotropic properties of biological tissue can be detected by magnetic resonance (*1–3*). For example, NMR parameters like signal intensity and transverse relaxation time T_2 vary with the angle between the preferential direction of tendon and the polarizing magnetic field $\mathbf{B}_0$. Evidence of this is given by studies of tendon from animal carcasses as well as of human tendon *in vivo* (*4–7*).

Tendon consists of collagen microfibrils composed of tropocollagen, that is, protein consisting of three polypeptide chains arranged in a triple-helix configuration. These microfibrils are organized into fibrils which form fibers embedded in amorphous base material (*4*). The dense collagen structure is the reason why little to no signal is observed from tendon in NMR imaging experiments. The anisotropic properties of tendon have led to some misinterpretations in medical magnetic resonance imaging. If signal was observed in tendon it has been considered as pathologic and indicative of, e.g., tendon degeneration, abnormal vascularity, connective tissue, tendonitis, or straight rupture (*4, 5*).

To properly assess the signal detected from tendon the fiber orientation must be taken into account. If the preferential orientation of tendon is parallel to the $\mathbf{B}_0$ field, a situation often encountered in medical NMR imaging of humans, little to no signal is observed with clinical imagers. But at an angle between 40° and 70° to the $\mathbf{B}_0$ field, for example for tendon in the shoulder, wrist, or ankle, an NMR signal can readily be measured. This effect is known as the magic-angle phenomenon (*4–6*). The tendon structure of ordered fibers is responsible for this anisotropic behavior of the NMR properties. In previous studies, an orientation dependence of T_2^{-1} was found to scale with the square of the second Legendre polynomial $\frac{1}{2}$ $(3 \cos^2 \theta - 1)$, which defines the orientation dependence of second-rank tensor interactions in solid-state NMR such as magnetic susceptibility and dipole–dipole interaction (*3*).

Because most current clinical NMR imagers do not allow for orientation-dependent studies with respect to $\mathbf{B}_0$, the magic-angle phenomenon is often considered to be a nuissance in interpreting NMR imaging results, and systematic *in vivo* investigations have not been carried out. However, such investigations can be conducted with single-sided NMR. One such device is the NMR-MOUSE (*8, 9*), a mobile universal surface explorer, which can be positioned on the object at various orientations of the $\mathbf{B}_0$ field relative to the tendon direction. This device is proposed as a diagnostic tool to investigate tendon with the potential of detecting degenerative changes and following postoperative recovery. Investigations of tendon from animal carcasses and *in vivo* measurements of the human Achilles tendon performed with the NMR-MOUSE are reported below.

RESULTS AND DISCUSSION

Three series of measurements were carried out. The first series was intended to validate findings reported in the literature (*4–7*) that the anisotropic structure of tendon is associated with an orientation dependence of the NMR parameter T_2 and to demonstrate that this effect can be measured with the NMR-MOUSE. Tendon from pig and cow carcasses was positioned on the surface of the NMR-MOUSE beginning at about 12 h after slaughter. The spin–spin relaxation time T_2 was measured at different angles between the preferential direction of the
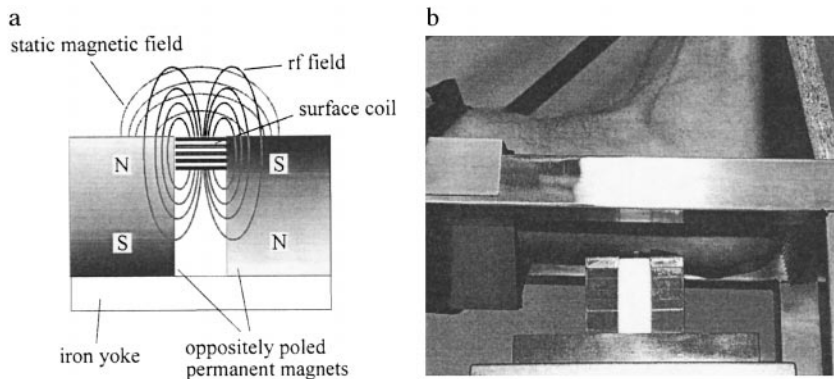
Ⓐℙ

FIG. 1. *In vivo* measurements of T_2 in Achilles tendon by the NMR-MOUSE. (a) The NMR-MOUSE provides the static magnetic field $\mathbf{B}_0$ by permanent magnets in u-shaped geometry. The rf coil is placed in the gap between the poles. The sensitive volume is located in the region above the device, where $\mathbf{B}_0$ and $\mathbf{B}_0$ exhibit orthogonal components. (b) Experimental setup for measurement of the Achilles tendon.

tendon and $\mathbf{B}_0$ in the range between 0° and 90° using the Carr–Purcell–Meiboom–Gill (CPMG) sequence (*10, 11*).

In a second set of experiments the relaxation anisotropy was investigated as a function of the tendon deterioration time. Tendon deterioration was achieved by leaving the material at room temperature. After a few hours, noticeable signs of tendon decay are a change of color and the development of an unpleasant smell. T_2 was monitored for pig tendon at 20, 43, 72, 94, and 165 h after acquisition of the material from the slaughter house. The time from slaughtering to the first measurement is estimated to be 12 h. For comparison, the same experiments have been conducted with cow tendon.

To demonstrate the feasibility of *in vivo* application of the NMR-MOUSE a third set of experiments was carried out with the human Achilles tendon in volunteers. The Achilles tendon can readily be detected with the NMR-MOUSE. Because skin and other tissue separate tendon and scanner surface, penetration depths of 3 to 4 mm had to be accessed in close contact of the scanner with the leg. For these investigations a larger version of the NMR-MOUSE was used with stronger magnets and a larger coil (Fig. 1). The spin–spin relaxation time T_2 has been measured once with the tendon parallel to the $\mathbf{B}_0$ field and a second time at an angle of about 55° between tendon and $\mathbf{B}_0$. The reproducibility of measurement was tested by repeating the experiments once after repositioning the leg. Further experiments at other angles could not be done without compromise to the comfort of the volunteers due to the duration of the measurements. For reference, the palm of each volunteer was measured to obtain data for isotropic tissue.

Figure 2 depicts the dependence of T_2^{-1} on the angle between the orientation of a pig tendon and $\mathbf{B}_0$ measured with the NMR-MOUSE. These results show a clear variation of T_2^{-1} with the orientation angle in agreement with observations reported in the literature (*1–7*). The relaxation rate constant shows a maximum value where the tendon orientation and $\mathbf{B}_0$ are parallel and a minimum between 50° to 60° near the magic

angle. In agreement with the literature, the angle dependence of $1/T_2$ follows the square of the second Legendre polynomial $\frac{1}{2}(3\cos^2\theta - 1)^2$. A preliminary account of this work has been given in Fig. 7 of Ref. (*9*) by a plot of T_2 versus θ.

In simple liquids, the nuclear dipolar interaction is averaged to zero due to the free mobility of the molecules. In tendon, the molecular mobility is restricted due to the confinements imposed by the ordered structure of the packed tendon fibers. As a consequence, the molecular mobility becomes anisotropic, and the nuclear spin interactions are averaged only partially and in an anisotropic fashion. The dipole–dipole interaction typically provides the strongest contribution to transverse relaxation. The residual dipole–dipole interaction of the protons in tendon follows the macroscopic order of the material and gives rise to the observed orientation dependence of the transverse magnetization decay.

Figure 3 illustrates the effect of tendon degeneration on the spin–spin relaxation rate constant. The transverse relaxation
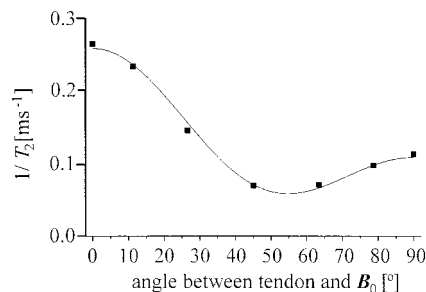

FIG. 2. Angular variation of $1/T_2$ for pig tendon. The angle measures the deviation of the tendon orientation from the direction of the magnetic field $\mathbf{B}_0$. The functional dependence of $1/T_2$ on the orientation angle can be described by the square of the second Legendre polynomial. The solid line represents the least squares fit of Eq. [1] to the experimental data points. The relaxation rates are slightly different from those in Fig. 2a because measurements were performed on different samples. A preliminary account of these measurements has been given by a plot of T_2 versus angle in Fig. 7 of Ref. (*9*).
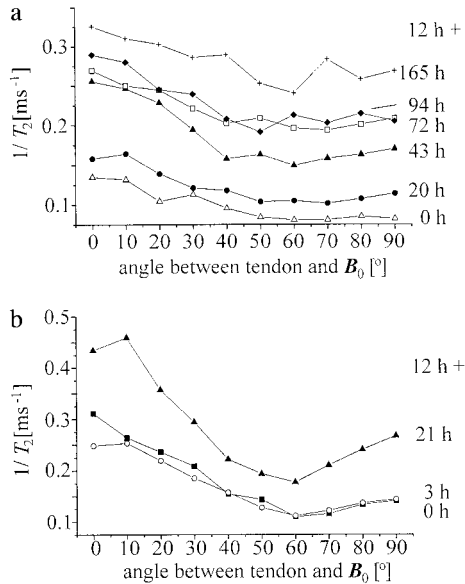
FIG. 3. The effect of aging on the angle-dependent transverse relaxation rate. The time from slaughtering to the first measurement (ca. 12 h) the tendon has been cooled in a refrigerator. In between measurements, the tendon was air-sealed at room temperature. With progressing decay of the tendon the oriented structure disintegrates and the orientation dependence of T_2 diminishes; (a) pig tendon; (b) cow tendon.

rate constants of tendon from pig and cow show a strong dependence on the orientation angle. Gauged by T_2 relaxation times, the tendon remains stable for a few hours. After about 30 h, including the 12 h from the death of the animal to the start of measurement, a considerable decrease in signal intensity and an increase in $1/T_2$ are observed.

As a measure of tissue orientation, the amplitude and the offset of the second Legendre polynomial can be employed. The offset $1/T_{2\mathrm{iso}}$ can be interpreted to characterize the isotropic tissue and the quantity $(1/T_{2\mathrm{aniso}})^{1/2}$ to characterize the anisotropic components of the tissue,

$$\frac{1}{T_2} \approx \frac{1}{T_{2\mathrm{iso}}} + \left(\frac{1}{T_{2\mathrm{aniso}}^{1/2}} \frac{1}{2} \left(1 - 3 \cos^2\theta \right) \right)^2. \qquad [1]$$

Figure 4 depicts the variations of $1/T_{2\mathrm{iso}}$ and $(1/T_{2\mathrm{aniso}})^{1/2}$ with the tendon disintegration time. From this observation a model of the disintegration process can be postulated. Taking the value of $(1/T_{2\mathrm{aniso}})^{1/2}$ as time invariant, the disintegration process resembles a melting process. With increasing time more and more tendon structures break apart into isotropic material while the remaining structures keep their full molecular order and thus their relaxation anisotropy. The increase in $1/T_{2\mathrm{iso}}$ is attributed to the disintegration process of the cell structures. The more cells disintegrate, the higher the isotropic relaxation rate constant.

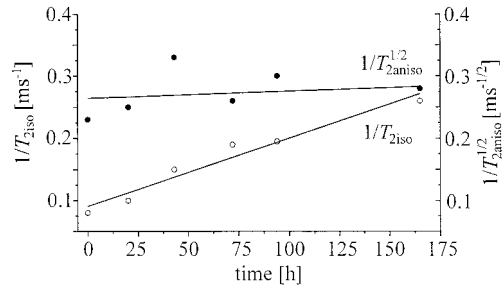Results from *in vivo* measurements of the Achilles tendon of

FIG. 4. Fit parameters $1/T_{2\mathrm{iso}}$ and $1/T_{2\mathrm{aniso}}^{1/2}$ of Eq. [1] for pig tendon (cf. Fig. 3a).

four volunteers are shown in Fig. 5. As in the studies of excised animal tendon a pronounced angle dependence is observed. The value of T_2 is low ($1/T_2$ is high) when the tendon is oriented parallel to the direction of the magnetic field. Near the magic angle of 54.7° the value of T_2 is approximately twice as high as at 0°. For comparison, the T_2 value of unorientated palm tissue is shown as well. Here no orientation dependence of T_2 is observed, indicating that the measured variation of T_2 from the Achilles tendon is indeed a phenomenon of macroscopic molecular order. Furthermore, the T_2 value of isotropic palm tissue agrees with the isotropic value of T_2 of tendon at the magic angle. A possible explanation is that in the soft palm tissue the nuclear spin interactions of the observed magnetization are isotropically averaged by fast molecular motion to the same isotropic value as in tendon. In the fast motion limit the quality of this agreement can then be a measure of the degree of molecular orientation in tendon: For partial orientation the magic-angle value for tendon should be lower than that for the isotropic mean. The difference between two measurements from the same person and at the same angle is in the range of the measuring error so that the measurements and can be considered reproducible. Significant contributions of the aniso-
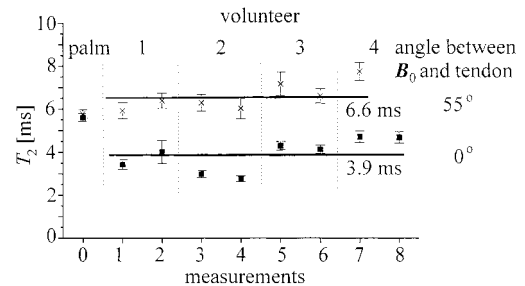

FIG. 5. *In vivo* measurements of T_2 in human Achilles tendon of four volunteers at orientation angles 0° and 55°. Each value of T_2 has been measured twice with repositioning of the foot in between measurements. For reference, the isotropic tissue of the palm has been measured with the same adjustments. The error bars indicate the accuracy of the T_2 values derived from a monoexponential fit to the measured data. One data point is missing for volunteer 4 due to discomfort of the volunteer associated with the duration of the measurement.

tropic magnetic susceptibility to the measured T_2 anisotropy resulting from the sample shape can largely be excluded, because the relaxation anisotropy was observed in Hahn-echo experiments, where magnetization dephasing from linear spin interactions is refocused.

It is fair to assume that the isotropic relaxation rate and the relaxation anisotropy probe the physical condition of tendon. But these parameters can only be a relative measure of tendon properties due to possible variations in different people. A correlation of tendon T_2 relaxation with age and physical condition of people still needs to be performed. Furthermore, T_2 may be a suitable parameter to follow postoperative recovery, for example, after a rupture.

CONCLUSIONS

Measurements with animal tendon show that the NMR-MOUSE is a device suitable to observe anisotropic T_2 relaxation of tendon. The advantage of using the NMR-MOUSE for that purpose is that the required variation of the angle between the tendon direction and the orientation of the magnetic field $\mathbf{B}_0$ is easy to achieve by simple rotation of the probe. Medical NMR imagers typically do not allow for this degree of freedom, so that anisotropy in tissue can be followed only by investigations of the anisotropy of translational diffusion (12, 13). The NMR-MOUSE provides an inexpensive alternative to some of these investigations.

The measurements of the human Achilles tendon prove that the NMR-MOUSE can be used for *in vivo* measurements. In the case of the Achilles tendon, the anisotropic behavior of T_2^{-1} and the magic-angle value can be a measure to assess the condition of tendon and to detect degenerative changes. Further investigations of patients with tendon dysfunction are required to assess how well this approach can be developed into a diagnostic tool. Shaping of the sensitive volume by magnet pole shoes and signal selection by pulsed field gradients in combination with selective pulses are conceivable improvements for suppression of nontendon signals.

EXPERIMENTAL

The NMR-MOUSE is an NMR sensor based on the principle of inside-out NMR (14). It consists of permanent magnets, which provide the $\mathbf{B}_0$ field, and a surface radio-frequency coil, which provides the $\mathbf{B}_1$ field (8). The sensitive volume of the NMR-MOUSE is located above the surface of the device. It is roughly ellipsoidal in shape. For measurement the probe is positioned on the sample surface or vice versa. The $\mathbf{B}_0$ field is highly inhomogeneous and decreases with increasing distance from the surface. This field variation enables the depth of the sensitive volume to be selected by variation of the resonance frequency. The NMR-MOUSE is connected to a PC-based spectrometer. The entire apparatus is a small mobile, stand-alone NMR device, which can be used without significant support and space requirements as opposed to conventional medical imagers.

For the investigations of excised tendon, a first-generation Maran PC spectrometer by Resonance Instruments was used. Two hundred CPMG echoes were recorded per scan, and the signals from 2000 scans were averaged for signal-to-noise improvement. The other acquisition parameters were echo time $t_E = 200$ μs, pulse lengths $t_{90} = 2.5$ μs and $t_{180} = 5$ μs, and transmitter power $P = 60$ W. The nominal 90° pulse is defined by the maximum signal obtained for two-pulse echo excitation by variation of the first pulse. The particular NMR-MOUSE used weighs less than 1.5 kg. For a sensitive volume at the surface of the scanner with a depth between 0 and 0.5 mm the ^{1}H resonance frequency was 17 MHz. This frequency was chosen for the measurements.

For the *in vivo* experiments a larger NMR-MOUSE was employed with a ^{1}H resonance frequency of 14.5 MHz at 3 mm depth. The investigations were carried out with the sensitive volume centered at 3 mm below the surface of the scanner. During measurement, the leg of a volunteer rested on a specially designed support to provide sufficient comfort and ensure immobility during the measurement time of roughly 10 min. Two hundred echoes were recorded with an echo time of $t_E = 200$ μs for each of the 5000 scans of a single measurement. A newer PC spectrometer ("the Minispec," Bruker Analytic GmbH) was employed, which permitted the use of amplitude modulation for flip angle variation so that equal pulse durations could be employed for the 90° and 180° pulses. In this case the excitation band width is the same for all pulses, and the signal-to-noise ratio improves because the size of the sensitive volume increases.

ACKNOWLEDGMENT

REFERENCES

1. H. J. C. Berendsen, Nuclear magnetic resonance study of collagen hydration, *J. Chem. Phys.* **36**, 3297–3305 (1962).

2. R. M. Henkelman, G. J. Stanisz, J. K. Kim, and M. J. Bronskill, Anisotropy of NMR properties of tissues, *Magn. Reson. Med.* **32**, 592–601 (1994).

3. Y. Xia, Relaxation anisotropy in cartilage by NMR microscopy (μMRI) at 14-μm resolution, *Magn. Reson. Med.* **39**, 941–949 (1998).

4. S. J. Erickson, I. H. Cox, J. S. Hyde, G. F. Carrera, J. A. Strandt, and L. D. Estkowski, Effect of tendon orientation on MR imaging signal intensity: A manifestation of the "magic angle" phenomenon, *Radiology* **181**, 389–392 (1991).

5. M. E. Timins, S. J. Erckson, L. D. Estkowski, G. F. Carrera, and R. A. Komorowski, Increased signal in the normal supraspinatus tendon on MR imaging: Diagnostic pitfall caused by the magic-angle effect, *Am. J. Radiology* **164**, 109–114 (1995).

6. G. D. Fullerton, I. L. Cameron, and V. A. Ord, Orientation of tendon in the magnetic field and its effect on relaxation times, *Radiology* **155,** 433–435 (1985).

7. Z. S. Rosenberg, Y. Cheung, M. H. Jahss, A. M. Noto, A. Norman, and N. E. Leeds, Rupture of posterior tibial tendon: CT and MR imaging with surgical correlation, *Radiology* **169,** 229–235 (1988).

8. G. Eidmann, R. Savelsberg, P. Blümler, and B. Blümich, The NMR MOUSE, a mobile universal surface explorer, *J. Magn. Reson. A* **122,** 104–109 (1996).

9. B. Blümich, P. Blümler, G. Eidmann, A. Guthausen, R. Haken, U. Schmitz, K. Saito, and G. Zimmer, The NMR MOUSE: Construction, excitation and applications, *Magn. Reson. Imaging* **16,** 479–484 (1998).

10. H. Y. Carr and E. M. Purcell, Effects of diffusion on free precession in NMR experiments, *Phys. Rev.* **94,** 630–635 (1954).

11. S. Meiboom and D. Gill, Modified spin-echo method for measuring nuclear relaxation times, *Rev. Sci. Instrum.* **29,** 688–691 (1958).

12. T. L. Chenevert, J. A. Brunberg, and J. G. Pipe, Anisotropic diffusion in human white matter: Demonstration with MR techniques *in vivo, Radiology* **177,** 401–405 (1990).

13. B. Geil, Measurement of translational molecular diffusion using ultrahigh magnetic field gradient NMR, *Concepts Magn. Reson.* **10,** 299–321 (1998).

14. R. L. Kleinberg, Well logging, *in* "Encyclopedia of Magnetic Resonance" (D. M. Grant and R. K. Harris, Eds.), pp. 4960–4968, Wiley, New York (1996).